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Lowering Plasma 1-Deoxysphingolipids Improves Neuropathy in Diabetic Rats

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Abstract:

1-Deoxysphingolipids (1-deoxySLs) are atypical neurotoxic sphingolipids which are formed by the serine-palmitoyltransferase (SPT). Pathologically elevated 1-deoxySL level cause hereditary sensory and autonomic neuropathy type 1 (HSAN1) an axonal neuropathy which is associated with several missense mutations in SPT. Oral L-serine supplementation suppressed 1-deoxySLs formation in HSAN1 patients and preserved the nerve function in an HSAN1 mouse model. As 1-deoxySLs are also elevated in patients with type 2 diabetes mellitus, L-serine supplementation could also be a therapeutic option for diabetic neuropathy (DN). This was tested in diabetic STZ rats in a preventive and therapeutic treatment scheme. Diabetic rats showed significantly increased plasma 1-deoxySL levels and L-serine supplementation lowered 1-deoxySLs levels in both treatment schemes ($p < 0.0001$). L-serine had no significant effect on hyperglycemia, body weight and food intake. Mechanical sensitivity was significantly improved in the preventive ($p < 0.01$) and therapeutic scheme ($p < 0.001$). NCV significantly improved in the preventive group only ($p < 0.05$). Overall NCV showed a highly significant ($p = 5.2E-12$) inverse correlation with plasma 1-deoxySL levels. In summary our data support the hypothesis that 1-deoxySLs are involved in the pathology of DN and that an oral L-serine supplementation could be a novel therapeutic option for treating DN.

Introduction

Diabetic neuropathy (DN) is one of the most important causes of morbidity and premature mortality in patients with diabetes (1; 2). It generates a great economic burden through its complications, including foot ulcers due to sensory loss and premature death due to dysregulated autonomic functions (3; 4). DN is a length-dependent axonal sensorimotor and autonomic neuropathy and usually starts in the lower extremities with either negative (e.g. numbness) or positive symptoms (e.g. neuropathic pain). Symptoms are mainly sensory and symmetrical on both sides with a “stocking and gloves” distribution. Yet, no adequate treatment for DN is available. Therapeutic options are restricted to symptomatic pain treatment with costly medications and potential side effects.

The underlying pathomechanisms leading to DN are not yet understood. Several histopathological changes are associated with DN including micro-angiopathy (5; 6), direct nerve injury and injury of the nerve supporting cells (e.g. Schwann cells in the PNS or satellite cells in the DRGs). Hyperglycemia is considered as an important contributor in the pathogenesis of DN (7-10) but even a tight control of hyperglycemia is not sufficient to prevent DN (3; 11-13). This suggests that other factors, apart from hyperglycemia, are underlying the pathogenesis of DN. Sphingolipids have been identified over the last years as emerging players in insulin resistance and T2DM (14). Sphingolipids are a heterogeneous class of lipids and essential components of the plasma membrane and plasma lipoproteins. The initial and rate-determining step in *de novo* synthesis of sphingolipids is the condensation of palmitoyl-CoA and the amino acid L-serine– a reaction which is catalyzed by the enzyme Serine-Palmitoyltransferase (SPT) (15). Several missense mutations in SPT cause the hereditary sensory and autonomic neuropathy type 1 (HSAN1), a rare length-dependent axonal neuropathy characterized by sensory loss, ulcers, and

autonomic dysfunction (16; 17). HSAN-1-causing mutations in SPT induce a gain of function by shifting the substrate affinity of the enzyme from L-serine to the non-canonical substrates L-alanine and glycine (18). This forms an atypical class of 1-deoxysphingolipids (1-deoxySL) which lack the C₁-hydroxyl group of the canonical sphingolipids. Consequently, 1-deoxySLs cannot be metabolized to complex sphingolipids nor degraded by the canonical pathway which requires the formation of sphingosine-1-phosphate as a catabolic intermediate. 1-deoxySL levels are significantly elevated in the plasma of HSAN1 patients as well as in plasma and nerves of a transgenic HSAN1 mouse model (18; 19). They are neurotoxic by inducing branching defects and neurite retraction in cultured primary DRG neurons (18). Interestingly, wild-type SPT is also able to metabolize L-alanine under certain metabolic conditions (20). We demonstrated in several clinical studies that 1-deoxySLs are significantly elevated in the plasma of individuals with MetS and T2DM (21; 22). Although the etiologies of HSAN1 and DN are different, the clinical presentation is rather similar. Both conditions are characterized by a painful, progressive and length-dependent late onset axonopathy which typically affects distal extremities first. The degeneration of small intra-epidermal sensory fibers results in the loss of pain sensation, which in turn leads to painless injuries and neuropathic pain attacks. Both, HSAN1 and DN are associated with painless skin ulcers and severely impaired wound healing which is not typically seen in other peripheral neuropathies. We previously showed that oral L-serine supplementation significantly reduces 1-deoxySLs level in the HSAN1 mouse models and patients (19). Moreover, L-serine-supplemented HSAN1 mice were protected from the development of neuropathy. On the basis of these findings, we were interested whether L-serine is also effective in lowering plasma 1-deoxySLs in context of diabetes and whether such a treatment could be a therapeutic option for DN.

Results

Serine-enriched diet increases plasma serine but does not affect body weight, hyperglycemia, hypertriglyceridemia or food intake in STZ rats.

A streptozotocin (STZ) induced diabetic rat model was used to test the effect of an oral L-serine supplementation on 1-deoxySL formation and DN. Two experimental designs were employed (Fig.1). In the preventive schedule, the animals had immediate access to either a serine-enriched or a standard diet. In the therapeutic schedule, animals remained on a standard diet for 8 weeks after STZ injection and were then randomized into groups receiving either a serine-enriched or a standard diet. STZ-treated animals developed hyperglycemia (Fig. 2A-C) within 48 hours post-injection. Hyperglycemia (400- 800 mg/dl or 22.2- 44.4 mmol/l) persisted in the STZ groups until the end of the study (preventive 18 weeks; therapeutic 24 weeks). Plasma triglycerides (TG) increased significantly in the diabetic animals reaching a maximum after 4 weeks (300-400 mg/dl or 3.38- 4.52 mmol/l) and remained elevated until the end of the study (Fig. 2D-F). Plasma serine levels were 4- 6 fold higher in the serine-supplemented animals (Fig 2G-I). This effect persisted until the end of the study. Serine supplementation did not affect hyperglycemia or hypertriglyceridemia (Fig2A-F) and had no influence on body weight, food or water consumption (Supplementary figure 1 A-I). In contrast to controls the STZ group failed to gain body weight despite the increased food and water intake (Supplementary figure 1 A-C).

Serine enriched diet lowers 1-deoxysphingolipids in the plasma of STZ rats without affecting other sphingolipids

Sphingoid bases in plasma are usually conjugated to a variety of N-linked fatty acids and different head groups. As we were primarily interested in the sphingoid base profile, the *N*-acyls and head groups were removed by acid hydrolysis. The profile of the free sphingoid bases was

analysed by LC-MS. Consequently, the herein reported sphingoid base concentrations reflect the total concentrations of sphingolipids which are formed on a specific sphingoid base backbone.

Sphingolipids containing a C₁₈SO backbone were the dominant forms in plasma and did not differ between the groups (Fig. 3 A-C). C₁₈SA levels were minor but increased initially in the serine-supplemented STZ animals, reaching a maximum after 3-4 weeks and then decreased again but remained slightly elevated until the end of the study (Fig.3 D-F). C₁₈-SA diene and C₁₈PhytoSO (Fig. 3 G-L) were significantly elevated in all diabetic groups but did not change upon serine supplementation.

Plasma 1-deoxySA was significantly elevated in the STZ rats on standard diet but remained low in the serine supplemented STZ rats. In the preventive schedule, the plasma 1-deoxySA was significantly higher in the STZ animals on standard diet ($p < 0.0001$) compared to the other groups (Fig. 4A-C). 1-deoxySA increased from $0.03 \mu\text{mol/l} \pm 0.003$ at the beginning of the study to reach $0.05 \mu\text{mol/l} \pm 0.007$ before sacrifice in the STZ rats on standard diet (Fig 3A, C) whereas the levels remained low in the serine-fed STZ rats until the end of the preventive scheme ($0.016 \mu\text{mol/l} \pm 0.001$ at week 17 post-STZ injection).

For the therapeutic scheme, we observed a rapid decrease of 1-deoxySA to control levels after switching to the serine-enriched diet in week 8 whereas 1-deoxySA levels remained high in the diabetic rats on standard diet (Fig. 4B). This effect remained significant until the end of the study (Fig. 4B). No significant difference in 1-deoxySA levels was observed between the control rats with and without serine supplementation.

Plasma levels of C₁₆SO (supplementary figure 2 A-C) did not differ significantly between the groups at baseline but were significantly reduced in the STZ groups in both scheme at the end of

the study. Plasma levels of C₁₇SO, C₂₀SO and C₂₀SA were not significantly different between groups and did not change upon treatment (Supplementary figure 2 D-L).

Interestingly, we could not detect significant 1-deoxySA levels directly in nervous tissues including sciatic nerve, spinal cord, dorsal root ganglia, of neither control nor of diabetic animals (Supplementary figure 3). Overall sphingoid bases distribution in the nervous tissues were similar between STZ and control animal. In comparison to plasma, nervous tissue had higher proportion of C₁₈SA and C₁₇SO and lower proportion of C₁₈SA diene.

Serine-enriched diet improves the diabetic neuropathy phenotype in STZ rats

STZ rats on standard diet showed a significant decrease in the force withdrawal threshold which was significantly improved in both L-serine supplemented groups (Fig 5A-C). In the preventive scheme, the force withdrawal threshold remained stable for the serine treated STZ animals (103.2 g \pm 11.6 at week 14) while the STZ rats on standard diet showed a continuous decrease over the whole study period (54.6 g \pm 5.0 at week 14). A significantly improved mechanical sensitivity was observed for the serine-treated STZ rats versus those on standard diet at the end of the preventive protocol ($p < 0.01$). No difference was seen between the control groups. Similar results were obtained in the therapeutic scheme. Mechanical sensitivity was not significantly different between the STZ groups at the start of the serine supplementation on week 8 (74.5 g \pm 6.6 in the STZ group on a standard diet and 73.4 g \pm 8.4 in the STZ group on a serine-enriched diet). However, L-serine supplementation improved mechanical sensitivity and the differences became significant at week 23 prior to sacrifice (52.0 g \pm 5.4 on standard diet and 101.8g \pm 13.0 on serine-enriched diet, $p < 0.001$) (Fig. 5C).

Thermal response latency (Fig. 5D-E) was not significantly different between control and STZ animals in the preventive group but reached significance in the therapeutic group (Fig. 5F). L-serine supplementation showed no significant effect on thermal response latency.

Nerve conduction velocity (NCV) decreased significantly in the STZ groups on the standard diet (Fig. 6 A) ($p < 0.0001$ comparing the STZ treated rats on standard vs control diet in the preventive schedule; $p < 0.001$ comparing the same groups in the therapeutic schedule). In the preventive scheme NCV was significantly improved in the L-serine supplemented STZ rats ($30.3 \text{ m/sec} \pm 2.2$ in the STZ rats on the serine-enriched diet vs. $23.8 \text{ m/sec} \pm 1.0$ for the STZ rats on the standard diet, $p < 0.05$). By trend NCV also improved in the therapeutic scheme but did not reach the statistical significance until the end of the study (Fig. 6A). Na^+/K^+ ATPase activity was significantly decreased in STZ rats on standard diet in both the preventive and therapeutic groups (Fig 6B) ($p < 0.001$ and $p < 0.01$, respectively). For both treatment schemes there was a trend for improved Na^+/K^+ ATPase activity upon serine supplementation but did not reach statistical significance after correcting for multiple comparisons. Intra-epidermal nerve fiber density was not different between control and diabetic rats (Fig 6C).

For all groups we observed a highly significant inverse correlation between plasma 1-deoxySA levels and NCV ($r = 0.62$, $p = 5.23 \times 10^{-12}$) (Fig. 6D).

Morphological and Morphometric analysis of sciatic nerve and DRG Nerve morphometry (Fig. 7A-D) indicated a change in the distribution of axon and nerve fiber diameters with less percentage of large diameter fibers. This was partly restored upon L-serine supplementation in the preventive but not in the therapeutic group. In the preventive group the distribution of axon and nerve fiber diameters was significantly different in the L-serine supplemented diabetic animal compared to those on standard diet ($p = 0.0001$ for the distribution of axon diameter and

$p < 0.0001$ for the distribution of nerve fiber diameter). This is mainly a result of the increase in percentage of axons with larger diameter $>7\mu\text{m}$ and a decrease in those with smaller diameter (3-7 μm). No significant difference in the distributions of axon/fiber diameter was seen upon serine supplementation for the therapeutic group. DRG neurons were smaller in size in the diabetic rats compared to controls and did not change upon supplementation (Fig.8 A). The morphometric analysis showed a significantly reduced somatic, nuclear and nucleolar size in the DRG neurons of the diabetic animals compared to controls independent of the diet (Fig.8 C-D). No evidence of cell damage was observed and satellite cells were normal in all groups

Discussion

We demonstrated previously that oral L-serine supplementation is effectively lowering 1-deoxySL plasma levels in HSAN1 animal model and patients (19). Here, we report that L-serine supplementation is also effective in reducing plasma 1-deoxySL levels in a diabetic STZ rat model. The reduced 1-deoxySL plasma levels were associated with improved sensory nerve function in the supplemented animals but had no effect on hyperglycemia or plasma TG levels (Fig.2 A-F). We found significant improvements in several neuropathy parameters including mechanical sensitivity, NCV, the percentage of large diameter fibers/axons and by trend an improved neuronal Na^+/K^+ ATPase activity. This indicates that lowering plasma 1-deoxySL levels is beneficial and protects from diabetes associated nerve damage. The negative influence of elevated plasma 1-deoxySL levels on nerve function is also supported by a highly significant negative overall correlation between plasma 1-deoxySLs and NCV.

STZ rats are generally considered to be a T1DM model which is, however, not fully correct as hyperglycemia in these animals is typically also early associated with dyslipidemia and elevated

plasma TGs (Fig.2 D-F). In T1DM patient DN often develops after a period of sustained or uncontrolled hyperglycemia (23) which coincides with dyslipidemia in these patients (24). In T2DM patients, dyslipidemia appears early and even precedes the onset of hyperglycemia. Hypertriglyceridemia has been shown to correlate with the progression of diabetic neuropathy independently of glycemic control (25; 26). In the European Diabetes Prospective Complications Study (EURODIAB), hypertriglyceridemia was identified as an independent predictor for the development of diabetic neuropathy in T1DM even after adjusting for the duration of diabetes and HbA1c (27). Plasma TG and 1-deoxySLs levels were shown to be independent variables but show a strong and highly significant correlation (21; 22). This correlation cannot be easily explained by direct metabolic interactions since 1-deoxySLs are formed by SPT due to a shift of the amino acid and not of the lipid substrate. In contrast to TGs whose plasma levels are in the millimolar range, 1-deoxySLs are present in plasma and neurotoxic *in vitro* in the low micromolar range.

The mechanisms through which 1-deoxySLs exert their neurotoxic effects are not yet understood. They impair length, number and branching of neurites in cultured dorsal root ganglia (DRGs) (28) and inhibit neurite growth and induced cytotoxicity in primary dopaminergic neurons (29). It was reported that 1-deoxySA can bind and activate endothelial differentiation gene (EDG) receptors in cell culture (30; 31). The EDG receptor family consists of several G protein coupled receptors that regulate various neuronal functions (32). Alternatively, 1-deoxySA may modulate protein kinase C (PKC) activity as it was shown previously for other free sphingoid bases (33-35). PKC is known to be involved in the pathogenesis of diabetic microvascular complications including diabetic neuropathy (36). Another line of evidence suggests that 1-deoxySLs impair neuronal cytoskeleton dynamics and growth cone formation

(18). It was shown that 1-deoxySA promotes the disassembly of actin stress fibers in Vero cells (37) and alters cytoskeleton dynamics in cultured INS-1 beta cells, resulting in the intracellular accumulation of filamentous actin, impaired insulin secretion and the activation of Rac1 (38). However, we cannot fully exclude that the observed beneficial effects of L-serine are also mediated by other, not yet defined, neurotropic mechanisms. Earlier reports showed that the addition of L-serine to embryonic chicken DRGs improves neuronal differentiation and survival in-vitro (39). Neurons cannot synthesize L-serine and therefore depend on the supply of serine from surrounding cells like glia and satellite cells. Further mechanistic studies are, therefore, necessary to dissect the interplay between 1-deoxySLs formation and the protective effect of serine in the context of DN. However, independent of the underlying mechanisms, our studies unraveled oral L-serine supplementation as a candidate treatment for diabetic neuropathy that merits further validation in clinical trials of patients with diabetes mellitus.

Materials and Methods

Animal experiments:

The Statement of Compliance (Assurance) with Standards for Humane Care and Use of Laboratory Animals has been reviewed (10/28/2008) and approved by the National Institutes of Health-Office for Protection from Research Risks (5023-01, expiration 10/31/2013). Male Sprague-Dawley rats (180-200 g, Charles River, Calco, Italy) were used for the study. The animals had access to food and water ad libitum. Diabetes was induced in overnight-fasted rats by a single intra-peritoneal injection of 60 mg/kg streptozotocin (STZ) (Sigma, St. Louis, MO) dissolved in sodium citrate buffer (pH 4.5). Control rats were injected with sodium citrate buffer (pH 4.5) only. Hyperglycemia was confirmed by measuring glycosuria 48 h after STZ injection (Keto-Diabur test, Roche Diagnostics, Spa, Italy). Blood glucose was determined after tail

bleeding using the Ascensia Elite assay (Bayer, Basel, Switzerland). Food and water intake were assessed at the specified time points by averaging over 2-days period. At the end of the study, animals were sacrificed; tissues were dissected and immediately frozen in liquid N₂.

Serine supplementation:

The animals had access to either a serine-enriched (containing 10% L- serine for the control group and 5% serine for the STZ group) or a standard diet (4RF21, Mucedola s.r.l, Milan, Italy). The serine content in the food for the STZ animals was half to compensate for doubled food intake of the STZ animals.

Behavioural tests and electrophysiology:

Thermal and mechanical nociception were assessed as behavioral measures for the diabetic neuropathy. The nociceptive threshold to radiant heat was quantified using the hot-plate paw withdrawal test (40). In brief, a 40-cm-high Plexiglas cylinder was suspended over the hot plate, and the temperature was maintained at 50°C to give a latency period of approximately 10 s for control rats. Withdrawal latency was defined as the time between placing the rat on the hot plate and the time of withdrawal and licking the hind paw (or manifesting discomfort). Mechanical allodynia on the plantar surface of the rat was assessed by a dynamic paw withdrawal test with a Dynamic Plantar Aesthesiometer (Ugo Basile, Comerio, Italy), which generates a linearly increasing mechanical force. The paw withdrawal reflex was recorded automatically by measuring the latency until withdrawal in response to the applied force.

Nerve Conduction Velocity (NCV):

NCV was measured as described previously (40). In brief, the anti-dromic sensory NCV in the tail nerve was assessed by placing recording ring electrodes distally in the tail. Stimulating ring electrodes were placed 5 and 10 cm proximally from the recording point. The latencies of the potentials, recorded at the two sites after nerve stimulation, were determined (peak to peak), and

NCV was calculated. All of the neurophysiological determinations were performed under standard conditions and at a controlled temperature (room and animals). Core temperature was maintained at 37°C by using heating pads and lamps.

Morphometric Analysis of Caudal Nerve

The caudal nerve was fixed in 3% glutaraldehyde, post-fixed in OsO₄, epoxy resin embedded and used for light microscopy and for morphometric analysis. Semi-thin sections (1 µm) were prepared, stained with toluidine blue and examined with a Nikon Coolscope light microscope (Nikon Instruments, Calenzano, Italy). For the morphometric analysis sections were analyzed in a photomicroscope (Nikon Eclipse E200; Leica Microsystems GmbH, Wetzlar, Germany) at a magnification of 60× and the morphometric analysis was performed using a QWin automatic image analyzer (Leica Microsystems GmbH, Wetzlar, Germany). All myelinated fibers in randomly selected sections from all specimens were counted and the external (total) and internal (axonal) diameters of myelinated fibers were measured (at least 500 myelinated fibers/nerves). From both axonal and total fiber diameters, the histogram of fiber distribution was calculated and the ratio between the two diameters (g-ratio) automatically calculated for each set of individual axon and fiber diameter. Histograms of the population distribution of myelinated fibers and axons, separated into class intervals increasing by 1.0 µm, were constructed.

Morphometric Analysis of DRG

At the end of the treatment period three L4-L5 dorsal root ganglia (DRG)/animals were collected and post-fixed in OsO₄, epoxy resin embedded and used for light microscopy. For each animal, several semi-thin sections (1 µm) were prepared from randomly selected blocks and were stained with toluidine blue then examined with a Nikon Coolscope light microscope (Nikon Instruments, Calenzano, Italy). The semi-thin sections were analyzed with a computer-assisted image analyzer

(Image J NIH software). The somatic, nuclear and nucleolar sizes of DRG sensory neurons were measured for at least 200 DRG neurons/animal in randomly selected sections.

All the morphometric measurements on caudal nerve and DRG were performed by the same examiner who was blinded regarding the belonging of the specimen to any experimental group.

Intra-epidermal nerve fiber density

Small fibers peripheral nerve damage was assessed by the quantification of the IENF density in the skin of the hind paw footpad (40). Hind paws were collected at sacrifice and 3-mm round-shaped biopsies, which included epidermis and dermis, were taken from the plantar glabrous skin and immediately fixed by immersion in 2% paraformaldehyde-lysine-periodate for 24 h at 4 °C, then cryoprotected overnight and serially cut with a cryostat to obtain 20 µm sections. Three sections from each sample were randomly selected and immunostained with rabbit polyclonal anti-protein gene product 9.5 antibodies (PGP 9.5; AbD Serotec, Kidlington, Oxfordshire, UK) using a free-floating protocol. One observer blinded to the status of the rats, independently counted the total number of PGP 9.5-positive IENFs in each section under a light microscope at high magnification. Individual fibers were counted as they crossed the dermal–epidermal junction or being located in epidermal layer. Secondary branching within the epidermis was excluded. The length of the epidermis was measured using a computerized system (Image-Pro Plus, Media Cybernetics, Inc., Silver Spring, MD, US), and the linear density of IENF was obtained.

Na⁺/K⁺ATPase activity:

Tibial stumps collected at sacrifice were dissected, desheathed and homogenized in chilled solution containing 0.25 M sucrose, 1.25 mM EGTA and 10 mM Tris, pH 7.5, at 1:20 (w/v), homogenized in a glass-glass Elvehjem-Potter (DISA, Italy) and stored at –80°C for ATPase

determinations. Na^+/K^+ -ATPase activity was determined spectrophotometrically as described previously (40). Protein content in homogenates was determined according to Lowry's with bovine serum albumin as standard.

Sphingolipid analysis:

The sphingoid base composition of the extracted lipids was analysed after hydrolysis as described before (22). Isotope labelled d7-sphingosine and d7-sphinganine (d7SA, d7SO; 200pmol; Avanti Polar Lipids, Alabaster, Alabama, US) was used as an internal standard. The sphingoid bases were separated on a C_{18} column (Uptisphere 120 Å, 5µm, 125 × 2 mm, Interchim, Montluçon, France) and analysed on a TSQ Quantum Ultra mass spec (Thermo, Reinach, BL, Switzerland). Each sample was measured as a singleton. Intra- and Inter-assay coefficient of variation (CV %) of the method was between 5% and 20%. The plasma and tissue levels of C_{16}SO , C_{17}SO , C_{18}SO , C_{18}SA , C_{18}SA diene, $\text{C}_{18}\text{PhytoSO}$, C_{20}SO , C_{20}SA , and 1-deoxySA sphingoid bases were quantified.

Amino Acid analysis:

Amino acids were analysed on a Zorbax Eclipse AAA column (150 x 4.5mm, 5µM, Agilent) according to manufacturer's instructions. A Shimadzu LC2010 HPLC system connected to a fluorescence detector (Hewlett Packard) was used for detection.

Triglyceride measurement:

Triglycerides were measured using an enzymatic assay Kit (Sigma-Aldrich, St. Louis, MO, US) according to the manufacture's protocol.

Statistical analysis:

Data are shown as mean ± SEM. For normally distributed variables, one way ANOVA is performed followed by the Bonferroni correction for the multiple comparisons. In the Bonferroni

correction, only four comparisons were considered, control on standard diet vs. control on serine diet; control on standard diet vs. streptozotocin on standard diet, control on serine diet vs. streptozotocin on serine diet and streptozotocin on standard diet vs. streptozotocin on serine diet. Variables which were not normally distributed were log-transformed. The statistical analysis was performed using SPSS 16.0 (IBM, Zurich, Switzerland) and GraphPad Prism 5.04 (GraphPad Software, Inc., San Diego, CA).

Author contributions:

AO was involved in the study design, did the lipid extraction, mass spectrometric analysis, triglyceride quantification, the statistical analysis and wrote the manuscript. IA and YW did the lipid extraction and tissue homogenization. . RB, CPS were involved in the study design, performed the animal experiment, phenotyping, neurobehavioral and neurophysiological tests. RL, CPS and CA helped RB in conducting the experiment, including diet administration and blood sampling. MC performed behavioral tests. ON performed neurophysiological tests (NCV). CG and GL were involved in the study design. AvE contributed to study design, data interpretation and critically revised the manuscript. TH performed the plasma serine measurements, was involved in study design, data interpretation and supervised the study.

Acknowledgments:

TH is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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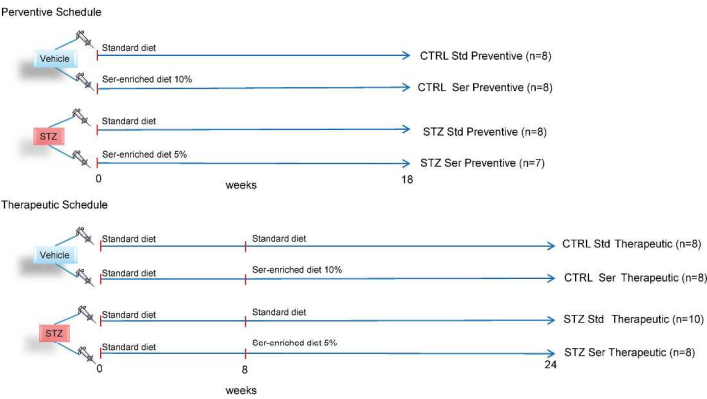
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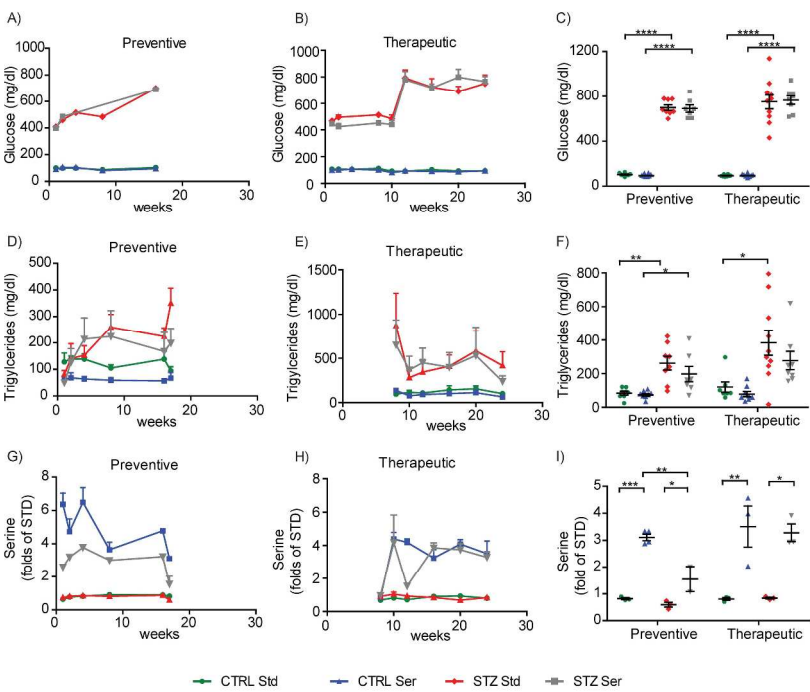
The authors declare no conflict of interest

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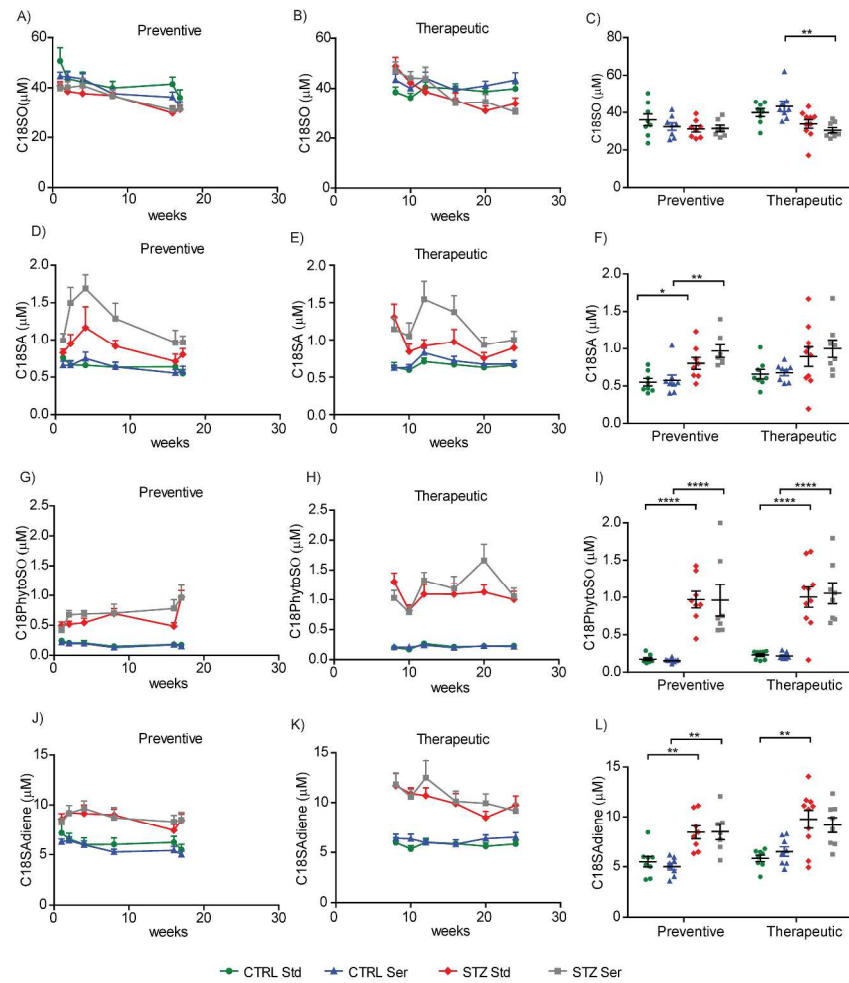
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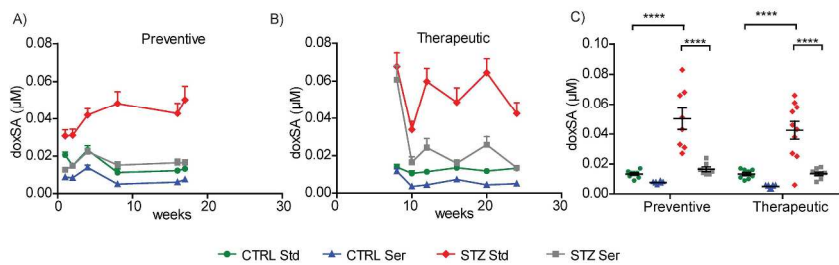


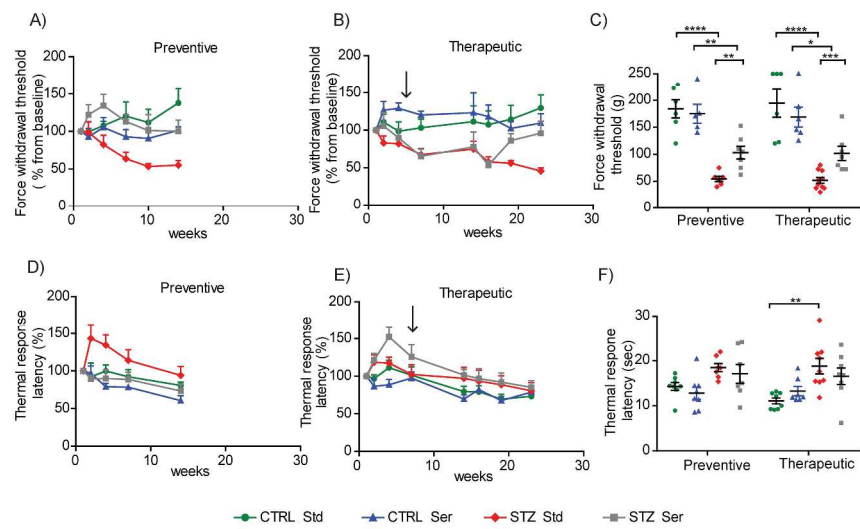


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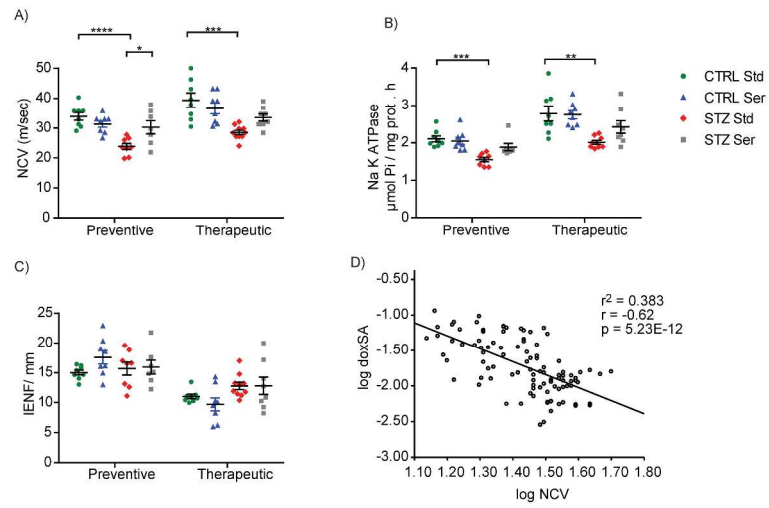


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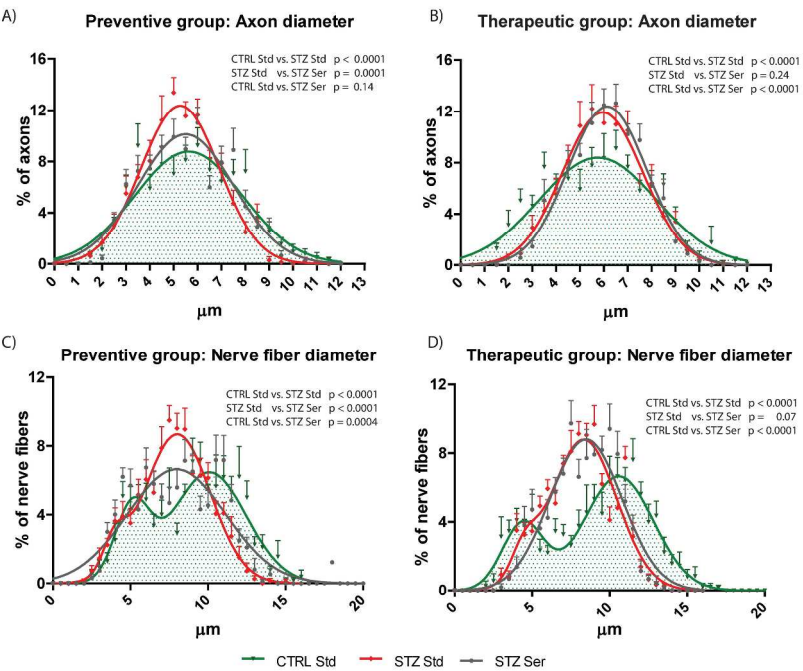




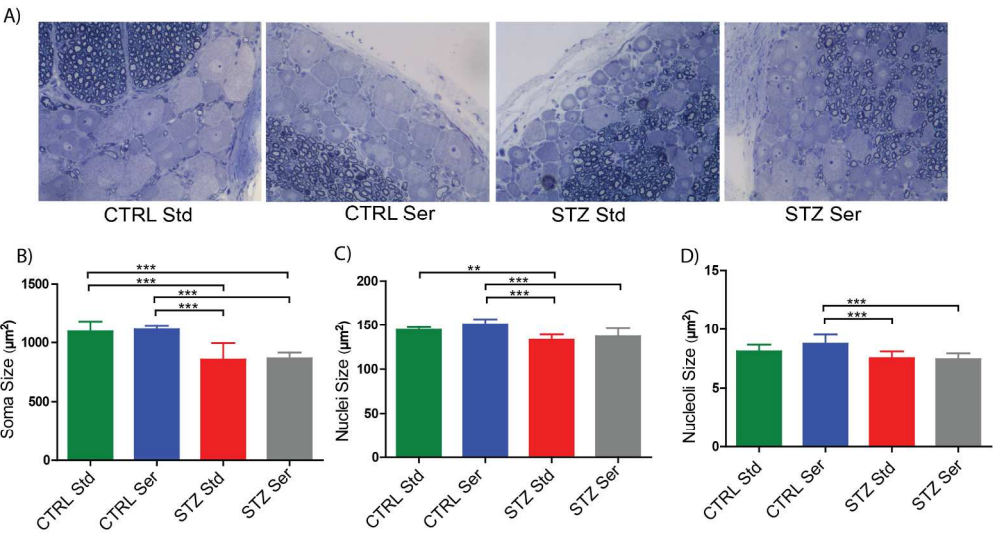
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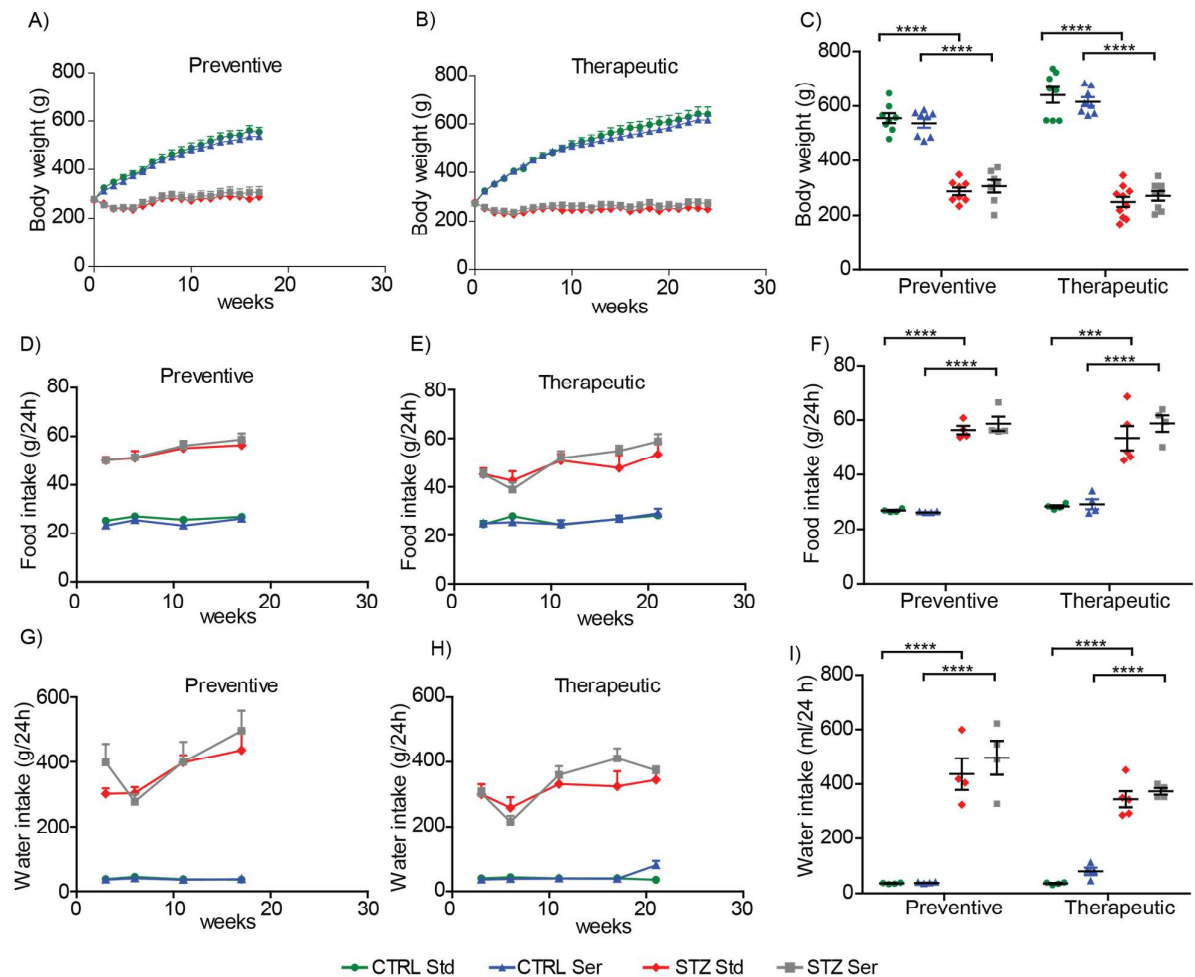
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FigureS1. Effect of serine on body weight, food and water intake. Line plots showing the body weight (A-B), blood glucose (C-D), food (E-F) and water intake (G-H) of the animal used in the study. The plots show the values over the entire period of the preventive (Left) and therapeutic (middle) schedules. Scatter plots show the values of the body weight (C), food (F) and water intake (I) at week 16 post-STZ injection for the preventive group and week 24 post-STZ injection for the therapeutic group. The values are expressed as mean \pm SEM. p values are calculated using ANOVA followed by the Bonferroni correction. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. CTRL Std, control on standard diet; CTRL Ser, control on serine diet; STZ Std, STZ on standard diet; STZ Ser, STZ on serine diet.

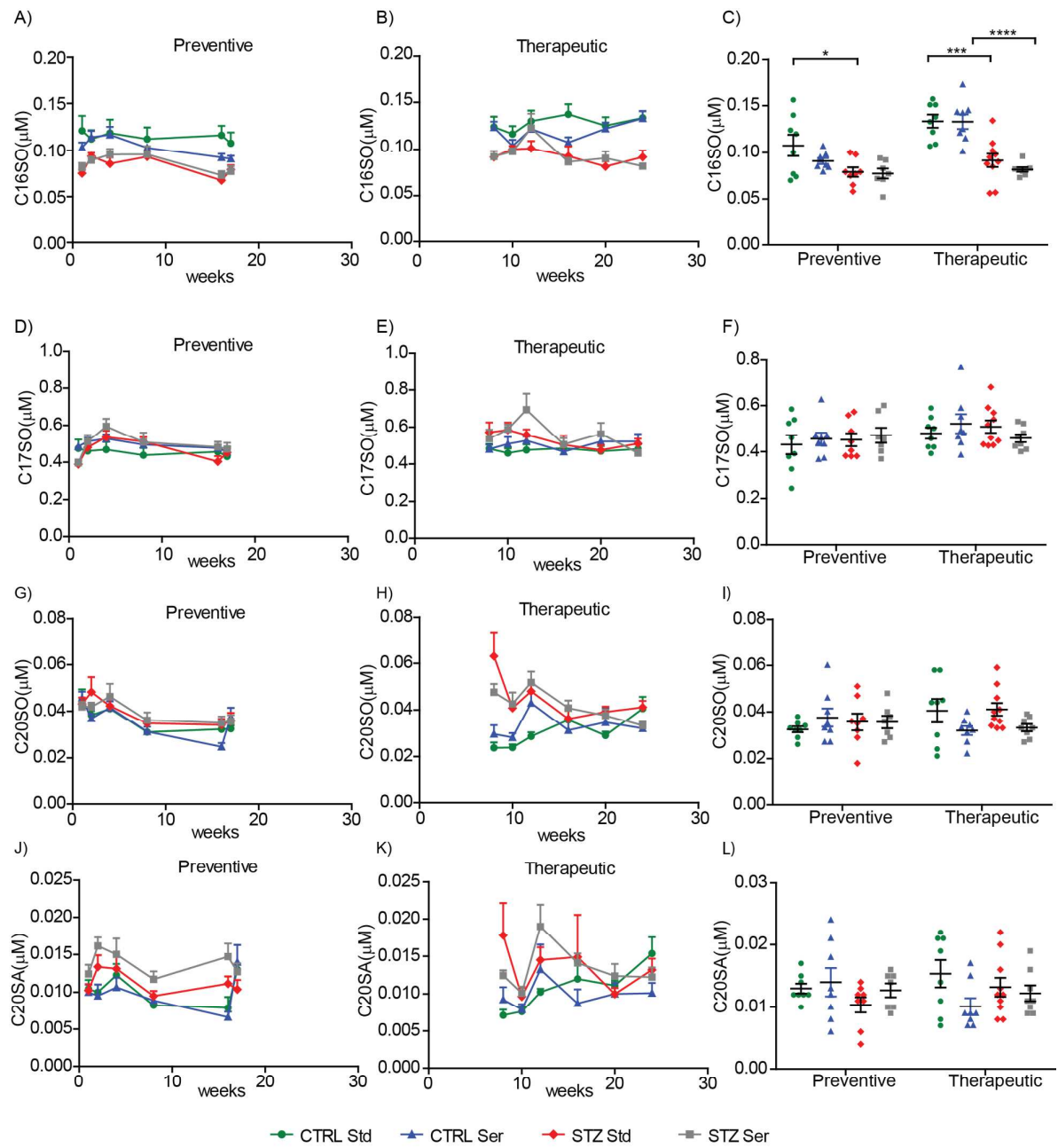


Figure S2. Effect of serine-enriched diet on plasma levels of atypical sphingolipids with different chain lengths. Line plots show plasma levels of C₁₆SO (A-B), C₁₇SO (D-E), C₂₀SO (G-H), C₂₀SA-based sphingolipids (J-K) for the preventive (Left) and therapeutic (middle) schedules over the study duration. Scatter plots show the values for C₁₆SO (C), C₁₇SO (F), C₂₀SO (I), C₂₀SA (L) at week 17 post-STZ for the preventive group and week 24 post STZ for the therapeutic groups. The values are expressed as mean \pm SEM. p values are calculated using ANOVA followed by the Bonferroni correction. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ CTRL Std, control on standard diet; CTRL Ser, control on serine diet; STZ Std, STZ on standard diet; STZ Ser, STZ on serine diet

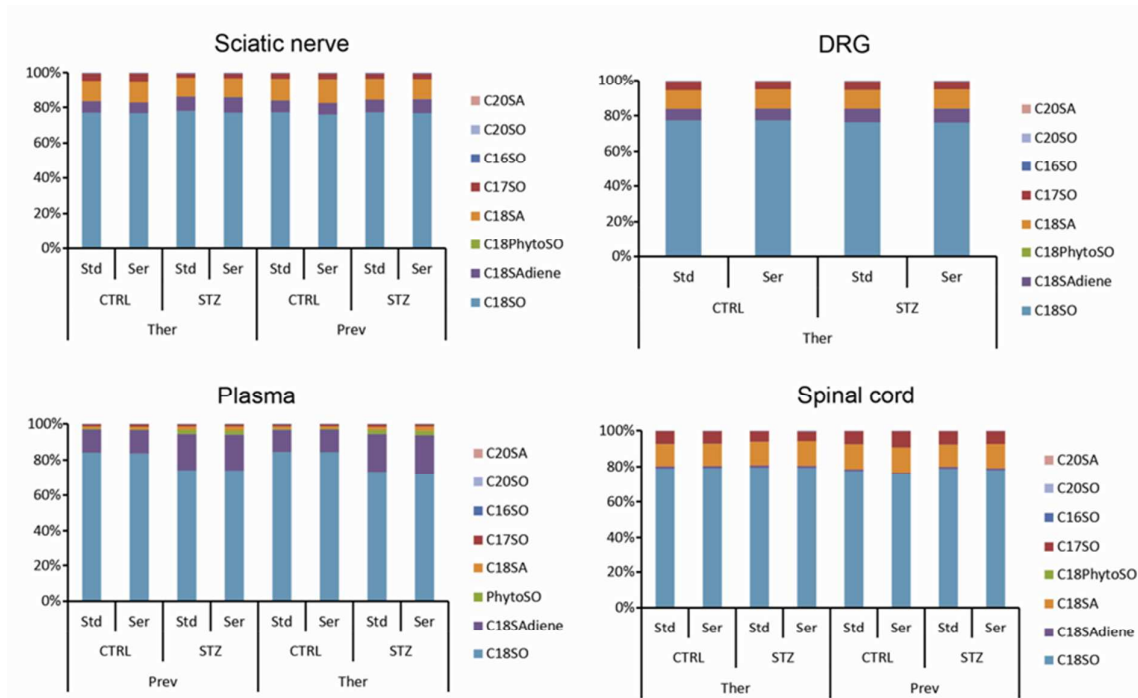


Figure S3. Sphingolipid distribution by backbone in the plasma, sciatic nerve, dorsal root ganglia (DRGs) and spinal cord. The total sphingolipid content is set to 100% and the relative ratio of each sphingoid base backbone is shown. CTRL, control animals; STZ, streptozotocin animals; Std standard diet; Ser, serine diet; Prev, preventive scheme; Ther, therapeutic scheme.